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SCIENTIFIC NOTE

HABITATS AND DISTRIBUTION OF *ANOPHELES SINENSIS* AND ASSOCIATED *ANOPHELES* HYRCANUS GROUP IN JAPAN

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ABSTRACT. Mosquito collections were carried out in August 2002 and July 2003 in Japan. *Anopheles sinensis* of the Hyrcanus Group, Myzomyia Series of *Anopheles*, was the most common species encountered. The distribution and habitats of 5 *Anopheles* Hyrcanus Group species are included. Eight species belonging to *Aedes*, *Culex*, and *Uranotaenia* were found associated with *An. sinensis* in rice paddies and a variety of other larval habitats.

KEY WORDS Mosquitoes, *Anopheles sinensis*, Hyrcanus Group, *Aedes*, *Culex*, *Uranotaenia*, Japan, Culicidae

The *Anopheles* Hyrcanus Group consists of several species that are vectors of malaria and other mosquito-borne diseases in the Oriental and Palearctic regions. The group, as currently defined, includes about three quarters of the species that comprise the Myzorhynchus Series of genus *Anopheles* subgenus *Anopheles*. The group has about 30 species, with the addition of 2 newly described species (Li et al. 2005, Rueda 2005) in the Oriental and Palearctic regions.

Anopheles hyrcanus (Pallas 1771) was initially recorded (as *Culex*) from the southern shores of the Caspian Sea. It was shown to have a wide distribution from Europe to east and southeastern Asia, including islands in the Indian and Pacific Oceans. It became apparent that the taxon included a number of morphologically distinct forms and that some were involved in the transmission of malarial and filarial parasites in the Oriental and Eastern Palearctic regions. Subsequently, about 30 species have been described and named (Ramsdale 2001, Harbach 2004, Rueda 2005). Harbach (2004) listed 27 species in the Hyrcanus Group, with 6 species placed in the Lesteri Subgroup, 4 in the Nigerrimus Subgroup, and 17 in the unassigned subgroup. He omitted *An. yatsushiroensis* Miyazaki and *An. albotaeniatus* (Theobald) from his Hyrcanus Group list, with the latter being transferred to the Albotaeniatus Group and the former currently a synonym of *An. pullus* M. Yamada (Shin and Hong 2001, Hwang et al. 2004). Twenty-seven species of the Hyrcanus Group are found in the Oriental and Eastern Palearctic regions, and only 3 species have a western Palearctic distribution (from western China and south of 50° N) (Ramsdale 2001). In this paper, we treated *An. yatsushiroensis* as a valid species because the genetic comparison to type specimens from the type locality of *An. yatsushiroensis*, Yatsushiro City, Japan, is still needed to rule out the possibility that this is a valid species.

In Japan, there are only 5 known species of the Hyrcanus Group: *An. sinensis* Wiedemann, *An. sineroides* Yamada, *An. engarensis* Kanda and Oguma, *An. lesteri* Baisas and Hu, and *An. yatsushiroensis* (Tanaka et al. 1979). The type locality of each species is listed in Table 1.

Anopheles sinensis is the most common anopheline species in Japan, including Ryukyu Islands (Tanaka et al. 1979). It has long been suspected that it is the most important vector of malaria in Japan, including Okinawa and Hokkaido. Even though indigenous malaria has disappeared, this vector remains abundant throughout Japan. It is a known vector of malaria in Korea and China, and it has a wide distribution in Asia (Harrison and Scanlon 1975, Tanaka et al. 1979, Rueda et al. 2005a). *Anopheles lesteri* is a very important vector of malaria in China. Based on a combination of published and newly generated rDNA ITS2 sequences, Wilkerson et al. (2003) found that *An. lesteri* from the Philippines (type locality of true *lesteri*) and Korea and so called *An. anthropophagus* from China are synonymous, with *An. anthropophagus* as the junior synonym. To clarify and stabilize the taxon, Rueda et al. (2005b) designated and described the

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Table 1. Type locality of *Anopheles* Hyrcanus Group species in Japan.

Species	Type locality	Reference
An. sinensis	China: Guangzhou	Wiedemann 1828
An. engarensis	Japan: Hokkaido (Engaru)	Kanda and Oguma 1978
An. yatsushiroensis	Japan: Kyushu (Yatsushiro)	Miyazaki 1951
An. sineroides	Japan: Hokkaido (Bibai)	Yamada 1924
An. lesteri	Philippines: Manila (Sta. Mesa)	Baisas and Hu 1936
	Laguna (Calauan)—neotype	Rueda et al. 2005b

neotype and alloneotype of *An. lesteri*. This species was suspected as an important vector of indigenous malaria in Japan, but particularly in Hokkaido, where it commonly occurs in great numbers. It is also common in the Ryukyus Islands and has been found more frequently in coastal regions than inland in Honshu and Kyushu (Tanaka et al. 1979). Shin and Hong (2001) and Hwang et al. (2004) considered *An. yatsushiroensis* as a synonym of *An. pullus* based on molecular and morphological data of Korean specimens. We did not collect *An. pullus* during our collections in 2002 and 2003 in Japan, and no report indicates the existence of *An. pullus* in that country. However, because the type locality of *An. yatsushiroensis* is in Japan, it is necessary to do a genetic comparison of *An. pullus* from Korea with the topotypic specimens from Japan to resolve definitively if the two are synonyms or not. *Anopheles yatsushiroensis* is not known as a vector of indigenous malaria in Japan. *Anopheles pullus* is considered a potential vector of *vivax* malaria in the Korean Peninsula. The other Hyrcanus Group species, *An. sineroides* and *An. engarensis*, are not known vectors of indigenous malaria in Japan.

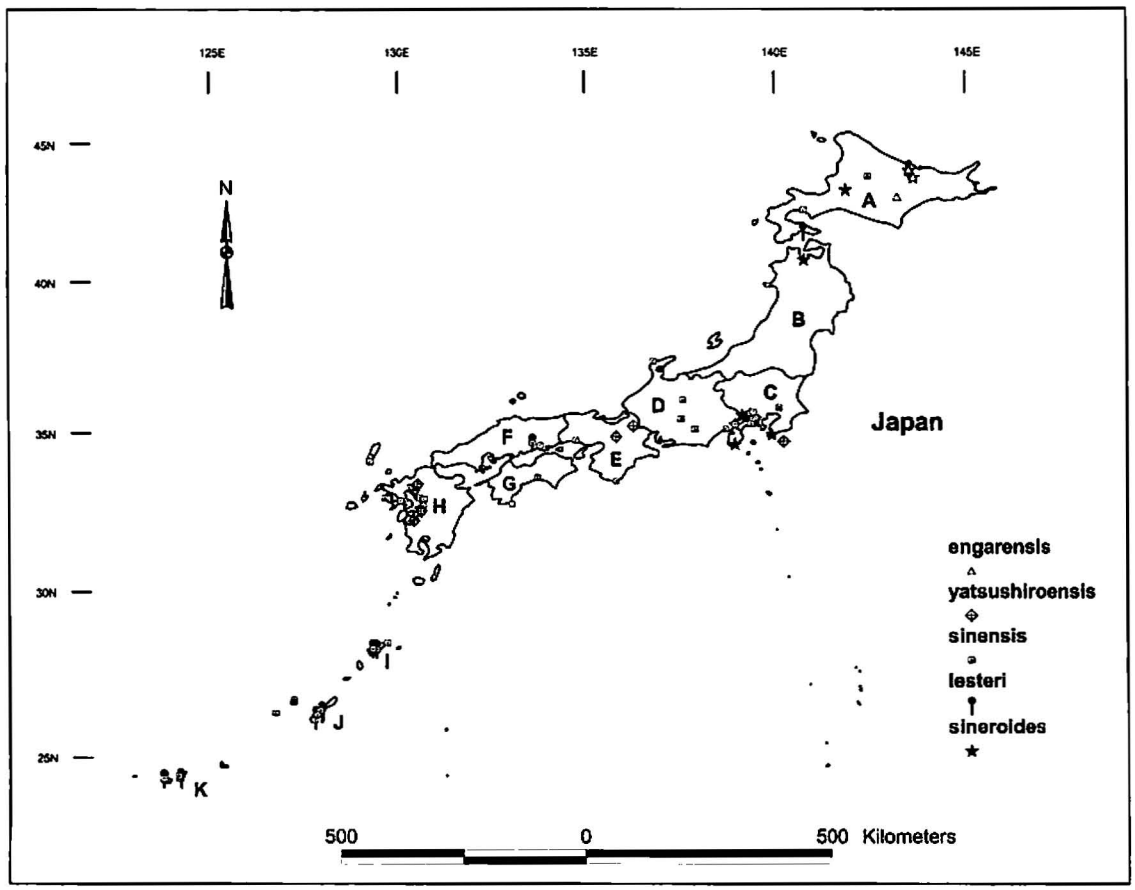

Fig. 1. Map of Japan showing the distribution of *Anopheles* Hyrcanus Group (based on observed and published specimens). (A) Hokkaido district. (B–F) Honshu. (G) Shikoku district. (H) Kyushu district. (I) Amami Guntô. (J) Okinawa Guntô. (K) Miyako and Yaeyama Guntô.

Table 2. Distribution of *Anopheles* Hyrcanus Group species in Japan (based on observed and published specimens).

Location	*An. engarensis*	*An. lesteri*	*An. sineroides*	*An. sinensis*	*An. yatsu-shiroensis*
Amami Oshima Is.: Akagina				6[1]	
Amami Oshima Is.: Koniya				1	
Amami Oshima Is.: Nishinakama		6		6	
Amami Oshima Is.: Yuwan		6, 7		6	
Hokkaido: Bibai			6	7	
Hokkaido: Engaru	6, 7	7	6, 7		
Hokkaido: Hakodate		1			
Hokkaido: Nagayama				7	
Hokkaido: Rubeshibe	6, 7		6	7	
Hokkaido: Sakura-oka			7	7	
Hokkaido: Sapporo	6				
Honshu: Aomori Pref., Sukayu			6, 7		
Honshu: Chiba Pref., Imba-Numa					2, 5
Honshu: Chiba Pref., Shirahama				1	
Honshu: Chiba Pref., Tateyama			1		
Honshu: Chiba Pref., Teganuma				6	
Honshu: Ishikawa Pref., Kamiosaki				1	
Honshu: Ishikawa Pref., Wajima				1	
Honshu: Kanagawa Pref., Atsugi			6, 7		
Honshu: Kanagawa Pref., Ayase			6		
Honshu: Kanagawa Pref., Hiratsuka				7	
Honshu: Kanagawa Pref., Nakatsu Valley				6	
Honshu: Kanagawa Pref., Sagamihara			6, 7	6	
Honshu: Kanagawa Pref., Toya			6, 7		
Honshu: Kanagawa Pref., Yamato			6, 7	6	
Honshu: Kanagawa Pref., Zama			6	6	
Honshu: Kyoto Pref., Hikone					5
Honshu: Kyoto Pref., Ogura					4, 5
Honshu: Nagano Pref., Norikura-kogen				6	
Honshu: Okoyama Pref., Kurashiki				6	
Honshu: Okoyama Pref., Utoma		6		6	
Honshu: Shizouka Pref., Gotenba			1	1	
Honshu: Shizouka Pref., Misakubo				6	
Honshu: Shizouka Pref., Shimoda			1		
Honshu: Tokyo Pref., Hachioji			6	6	
Honshu: Tokyo Pref., Kamiosaki				1	
Honshu: Tokyo Pref., Seroganeshizenen				1	
Honshu: Tokyo Pref., Shakuji				1	
Honshu: Tokyo Pref., Tachikawa				6	
Honshu: Tokyo Pref., Ueno			6	6	
Irimote Is.: Itokawa-rindo				6	
Irimote Is.: Mt. Bama		6			
Irimote Is.: Ohara				6	
Irimote Is.: Uehara		6			
Ishigaki Is., Inoda				6	
Ishigaki Is.: Miyara Riv.		6			
Ishigaki Is.: Mt. Maeshi				6	
Ishigaki Is.: Mt. Omoto		6		6	
Ishigaki Is.: Nagura				6	
Ishigaki Is.: near Kabira				6	
Ishigaki Is.: Yashigawa		6			
Ishigaki Is.: Yoshiwara				6	
Kyushu: Fukuoka Pref., Yanagawa					3
Kyushu: Kagoshima Pref., Kagoshima				6, 7	
Kyushu: Kagoshima Pref., Shiroyama				6	
Kyushu: Kagoshima Pref., Taniyama				6	
Kyushu: Kumamoto Pref., Minamata					3
Kyushu: Kumamoto Pref., Nishisato				7	
Kyushu: Kumamoto Pref., Tamana				6, 7	
Kyushu: Kumamoto Pref., Ueki				7	
Kyushu: Kumamoto Pref., Uto				6	
Kyushu: Kumamoto Pref., Yatsushiro				6, 7	3

Table 2. Continued.

Location	*An. engarensis*	*An. lesteri*	*An. sineroides*	*An. sinensis*	*An. yatsushiroensis*
Kyushu: Nagasaki Pref., Aino				6	
Kyushu: Nagasaki Pref., Fukushima Is.				7	
Kyushu: Nagasaki Pref., Isahaya				6	
Kyushu: Saga Pref., Ahikari				7	
Kyushu: Saga Pref., Saga and south of Saga				7	3
Kyushu: Saga Pref., Tosu				6	
Kyushu: Wakayama, Kushimoto				1	
Okinawa Is.: Ginowan		6			
Okinawa Is.: Igei				7	
Okinawa Is.: Kena Dam				7	
Okinawa Is.: Kin				7	
Okinawa Is.: Ojina		6, 7			
Okinawa Is.: Toguchi				6	
Okinawa Is.: Yona				6	
Shikoku: Kochi, Ashizuri-misaki				6	
Tsushima Is.: Asamo				6	
Tsushima Is.: Kuwa				6	
Tsushima Is.: Naiin				6	
Yonaguni Is.: near Sonai		6		6	

[1] 1 = Kurihara (1999); 2 = Miyake (1950); 3 = Miyazaki (1951); 4 = Otsuru and Konoe (1951); 5 = Otsuru and Ohmori (1960); 6 = Tanaka et al. (1979); 7 = observed specimens (museum and field collected).

We conducted 40 mosquito collections in Japan in August 2002 and July 2003, resulting in 223 individually reared pinned adults, 138 individually reared adults preserved in 100% ethyl alcohol for molecular tests, and 351 with exuviae of larvae and pupae and whole larvae. In addition, 3 type localities in Japan were visited to collect mosquitoes in 2003, namely Yatsushiro for *An. yatsushiroensis*, Bibai for *An. sineroides*, and Engaru for *An. engarensis*. In 2002, only the type locality in Yatshushiro was visited for collection. We initially targeted larval collections from those habitats where mosquitoes in the genus *Anopheles* were likely to be found. Larvae and pupae from various habitats were collected using dippers and individually reared to the adult stage (Walter Reed Biosystematics Unit 2001). Larval and pupal skins were preserved in 80% ethyl alcohol and slide mounted using standard protocols. Emerged adults were pinned, provided with appropriate collection data, and identified. Some adults and whole larvae were separately preserved in 100% ethyl alcohol for molecular analysis. Adult specimens and associated larval/pupal exuviae were identified using information from Tanaka et al. (1979). Voucher specimens are deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC.

Specimens of *Anopheles* Hyrcanus Group species deposited at various museums were examined, including those in the Entomology Museum of the National Institute of Infectious Diseases, Tokyo, Japan; University of Hokkaido, Insect Museum, Sapporo, Japan; and the National Museum of Natural History, Smithsonian Museum, Washington, DC. To further confirm the identification of 2 *Anopheles* species (i.e., *An. sinensis* and *An. sineroides*), DNA was isolated from individual adult mosquitoes by

Table 3. Habitats of *Anopheles* Hyrcanus Group species in Japan and other Asian countries.

Species	Larval habitat[1]	Locality	Reference
An. sinensis	RP, SP	Japan	Tanaka et al. 1979,[2]
	ID, MA, SW	Thailand	Harrison and Scanlon et al. 1975
An. engarensis	CR, GP, PO, RP, GP	Japan	Tanaka et al. 1979
An. yatsushiroenses	CR, ID, RP	Japan	Tanaka et al. 1979
An. sineroides	GP, PO, RD, SM	Japan	Tanaka et al. 1979[2]
	RH	Korea	Tanaka et al. 1979
An. lesteri[3]	GP, IW, MA, PO, RP, SM	Japan	Tanaka et al. 1979[2]
	HD	Philippines	Rueda et al. 2005b[2]

[1] CR, creek; GP, ground pool; HD, hill ditch; ID, irrigation ditch; IW, impounded water; MA, marsh; PO, pond; RD, road and other ditches; RH, rock hole; RP, rice paddy; SM, stream margin; SP, stream pool; SW, swamp.

[2] Observed museum and field-collected specimens.

[3] Prefer cooler and shaded areas unlike *An. sinensis*, and more common in coastal areas than inland (Tanaka et al. 1979).

phenol-chloroform extraction as described in Wilkerson et al. (1993). Direct sequencing of ribosomal DNA ITS2 was carried out, and the results were compared with those of Wilkerson et al. (2003).

Figure 1 and Table 2 show the distribution of 5 *Anopheles* Hyrcanus Group species, based on our field-collected data, museum specimen data, and published information (Miyake 1950, Miyazaki 1951, Otsuru and Konoe 1951, Otsuru and Ohmori 1960, Tanaka et al. 1979, Kurihara 1999). *Anopheles sinensis* occurs almost all throughout Japan (Honshu, Shikoku, and Kyushu districts and Rukyu Archipelago). *Anopheles lesteri* is found from the southern areas (Ryukyu Archipelago) to the northern areas (e.g., Hokkaido). *Anopheles yatsushiroensis* is known to occur primarily in the Honshu and Kyushu districts. *Anopheles engarensis* is confined to Hokkaido, whereas *An. sineroides* occurs in Hokkaido and other areas of Japan.

Table 3 shows the habitats of *An. sinensis*, *An. sineroides*, *An. engarensis*, *An. lesteri*, and *An. yatsushiroensis* at various areas of Japan based on our field-collected data, museum specimen data, and published information. The larvae of all species, except *An. sineroides*, are found in rice paddies. During our 2003 survey, we found that the mean water pH (6.8), conductivity (0.17 μS), and salinity (0.07 ppm) of the larval/pupal habitats of *An. sinensis* and associated species in rice paddies were greater compared with the irrigation ditches (6.5, 0.07, and 0.03, respectively). The mean water temperature (27.5°C) of these rice paddy habitats was lower compared with the irrigation ditches (30.5°C). We found the larvae of *An. sinensis* associated in the rice paddies with *Culex* (*Culex*) *tritaeniorhynchus* Giles at Saga (Saga Prefecture) and Yatsushiro (Kumamoto Prefecture), in the rice paddies with *Cx.* (*Cux.*) *bitaeniorhynchus* Giles at Nishisato (Kumamoto Prefecture), and in the taro or "rotas" irrigated fields with *Cx.* (*Lophoceraomyia*) *infantulus* Edwards, *Cx.* (*Cux.*) *tritaneorhynchus*, *Cx.* (*Lutzia*) *fuscanus* Wiedemann, and *Uranotaenea* (*Uranotaenea*) *macfarlanei* Edwards at Kin, Okinawa. We also collected from other larval habitats where *Anopheles* species were absent and found the following species: *Aedes* (*Aedimorphus*) *vexans nipponii* (Theobald) in rice paddies at Kin, Okinawa; *Cx.* (*Cux.*) *tritaneorhynchus* and *Cx.* (*Eumelanomyia*) *hayashii ryukyuanus* Tanaka, Mizusawa, and Saugstad in creeks or ditches at Camp Gonsalves, Okinawa; *Cx.* (*Eumelanomyia*) *hayashii hayashii* Yamada in rice paddies at Asahikawa (Hokkaido); *Cx.* (*Cux.*) *pipiens pallens* Coquillett and *Cx.* (*Lop.*) *infantulus* in stream margins at Sapporo (Hokkaido); and *Cx.* (*Cux.*) *mimeticus* Noe in rice paddies at Futoni and Bibai (Hokkaido).

This paper presents the habitats and distribution of the 5 species of Hyrcanus Group of *Anopheles* in Japan based on specimens collected in 2002 and 2003, museum specimens, and available references. Our work provides readily available information to preventive medicine and public health personnel who deal with malaria vector problems if sporadic malaria cases appear in the future.

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